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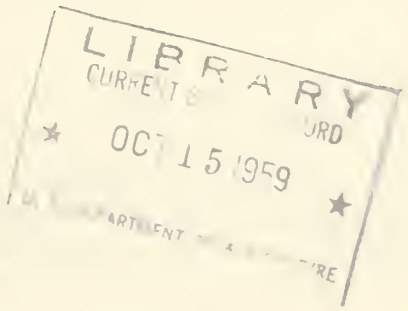
Substances Screened for Ability to Reduce Thermal Resistance of Bacterial Spores



Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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This publication is a supplement to "Search for Substances which Reduce the Heat Resistance of Bacterial Spores" by Michener, Thompson and Lewis, published in Applied Microbiology, Vol. 7, No. 3, 1959.

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UNITED STATES DEPARTMENT OF AGRICULTURE

SUBSTANCES SCREENED FOR ABILITY TO REDUCE THERMAL RESISTANCE OF BACTERIAL SPORES

H. David Michener, P. A. Thompson, and J. C. Lewis

The substances listed here were screened in order to find those which will reduce the thermal resistance of bacterial spores. The results are summarized and discussed, with methods and additional data on the activity of different concentrations of the more active substances, in Applied Microbiology, Vol. 7, no. 3, 1959 (Michener et al., 6). The complete list of screening tests on about 650 substances is presented here, together with methods and results of all tests. Although the ultimate objective of this work is the reduction of the thermal process required for sterilization of canned foods, many substances were selected for screening without special regard to their suitability for food use.

The test methods were designed for rapid screening. Each test is a comparison of the number of spores surviving a particular heat treatment in the presence and in the absence of the substance under test. Except in the preliminary testing, the results are expressed as the percentage reduction caused by the substance in the D value of the spores. The D value is the time required (under particular conditions of temperature, medium, and lot of spores) to kill 90% of the spores.

Thermal death time (TDT) tubes (9 x 120 mm.) were filled with 2 ml. of broth containing the substance to be tested, and inoculated with 6×10^4 spores of the Clostridium P.A. 3679. The broth was Andersen's (2) pork-pea medium, without thioglycollate, with 0.1% instead of 1.5% agar, and adjusted to pH 7.0. For each additive, a set of 4 or 5 tubes was made. These were heated in an oil bath at 113°C. for 43 minutes. This heat treatment was near the maximum that would allow spore survival and subsequent growth in all the control tubes. After the heat treatment, the tubes were incubated at 30°C. for 8 weeks and observed for growth. Under these conditions, any additive which significantly reduced the heat resistance of the spores caused the death of all of the spores in the tubes. The test was recorded as positive if half or more of the tubes failed to show growth (see Table 2, "Incubation Test").

This test was not sufficiently selective, presumably because bacteriostatic and sporostatic substances also gave a positive test. It was abandoned in favor of the "Counting Test" (Table 2). Substances which had given a negative incubation test (permitted growth) were usually assumed to be without effect on heat resistance and were not retested.

In the "Counting Test", tubes were prepared similarly (but, in duplicate) and inoculated with 6×10^5 spores. Each run usually contained 12 test substances and three pairs of controls. After they were sealed, the tubes were heated at 113°C. for 14 minutes, which permitted survival of about 5% of the spores in the controls. The tubes were then cooled, opened, diluted, and plated to count the surviving spores, using Andersen's (2) anaerobic plating technique.

From the number of surviving spores in the controls and in the tubes containing each additive, the reduction in D value was determined graphically for each additive (see Michener et al., 6). Most of the active substances were tested again in the same way but with a 10-minute heat treatment. This permitted survival of about 25% of the spores in the control tubes and a more accurate estimate of the reduction in D value when it was in the range of 50 to 90%.

The medium in the TDT tubes, together with the substance being tested, was diluted at least 1:1000 when plated. (Counts were not attempted at lower dilutions than this.) Separate tests showed that at this dilution the test substance affected spore outgrowth only for the few substances so marked in Table 2.

Substances were dissolved or dispersed in water or 0.025 M phosphate buffer at 10 x the concentration to be tested, and then were adjusted to approximately pH 7. A considerable number would not dissolve at this concentration. It was often possible to prepare uniform emulsions or suspensions by adding 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate, Atlas Powder Co.; inactive by itself). In a few cases, the test substance was first dissolved in a small amount of propylene glycol or ethyl alcohol, which was then diluted with water or a solution of 0.1% Tween 20. Organic solvents were used only at a final concentration known to be inactive in the test. Some substances were dissolved or dispersed with the aid of heating at 85°C. for a few minutes.

Some of the substances tested were not completely in solution even after the stocks were diluted 10-fold with broth. The activity recorded in such cases may have been only that of a saturated solution. However, the activity of certain antibiotics having a low solubility in water has been shown to increase as their concentrations are increased beyond that of saturated solutions (Michener et al., 5).

The spores were grown by L. E. Sacks of this laboratory on a medium containing the following, per l.: pork infusion, 400 ml.; pea infusion, 100 ml.; Difco Beef Extract, 10 g.; Difco Egg Albumin, Coagulated, water-soluble portion of 10 g.; NZ-Amine, 10 g.; Difco Proteose-Peptone No. 3, 10g.; Difco Yeast Extract, 4 g.; Difco Tryptone, 4 g.; soluble starch, 1 g.; sodium thioglycollate, 0.1 g.; KH_2PO_4, 3 g. The pH was adjusted to 7.2. Pea and pork infusions were prepared as described by Andersen (2). This medium was inoculated with an 18 hr. culture of P.A. 3679 and incubated at 25°C. for 15 days. The resulting spore suspension, containing about 3×10^7 spores per ml. of culture liquor, was stored at 2°C. Recently Zoha and Sadoff (7) have reported a simpler liquid medium suitable for production of spores of this organism.

Of the substances listed in Table 2, 67 were previously tested by Lewis et al (3). These and a few others were tested with a thermoresistometer, with P.A. 3679 spores suspended in pea puree, usually at a concentration of 14 ppm. The data from these tests are listed as "Thermoresistometer Test" (Table 2). These substances were retested only when the thermoresistometer

test gave a D value reduction of 8% or more.

The substances have been classified as indicated in Table 1. The classes are not mutually exclusive and a few substances are assigned to two or three classes. The abbreviations under "Class" (Table 2) refer to the classes listed in Table 1.

The statistical summary of results (Table 1) shows that about 10% of the substances reduced the D value of these spores by at least 20%, and about 4% of the substances reduced the D value by 45% or more. Activity is encountered frequently in some classes of substances and not at all in others.

In general, these substances are commercially available if their source is not given in the list of substances tested.[1/] The companies and individuals who supplied non-commercial samples for testing are listed in Table 3. The authors wish to thank them and acknowledge their help in pursuing this program.

1/ Mention of manufacturers or commercial products does not constitute endorsement by the U.S. Department of Agriculture over any similar product not mentioned.

Table 1. Summary of Screening Tests by Class of Substances

Class abbreviation	Class [a]	Number of substances which reduced D value by: Under 20% (inactive)	20 to 45%	Over 45%
A	Antibiotics [b]	177	3	4
AC	Chemicals stated to have antibiotic activity [c]	162	14	14
C	Miscellaneous chemicals	158	6	1
AA	Amino acids	8	0	0
Dis	Disinfectants	4	0	3
Dye	Dyes	3	2	0
FA	Fatty acids	7	4	0
E&G	Esters and glycerides of fatty acids	21	0	0
Su	Sugars	6	0	0
C&SA	Other carbohydrates and sugar alcohols	7	0	0
M	Mutagenic substances	26	10	10
Pl. ex.	Mixtures extracted from plants	7	0	1
SGS	Spore germination stimulants	2	0	0
So	Solvents	4	0	0
S	Sulfur compounds [d]	13	6	6
	All substances	588	35	26

[a] A few substances are included in more than one class.

[b] Including a few substances isolated from the higher plants.

[c] Chemicals described as having antibacterial, antifungal, antiviral, or antitumor activity; analogs of such chemicals; groups of chemicals prepared in order to test them for such activity.

[d] Not including antibiotics or sulfates.

Table 2. Results of Screening Tests

Class	Substance	Conc.[a] ppm,	Inhibition of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
Pl. ex.	Abies lasiocarpa, oil of	500	-		
M	Acenaphthene	500	+		0
FA	Acetate, sodium	500	+		-3
So	Acetone	1%			-19
C	Acetyl-o-aminophenol	500	-		
C	Acetylbenzoyl	500	+		11
C	Acetylcaproyl	500	+		17±
C	N-Acetylethanolamine	500	-		
C	Acetylsalicylic acid	500	-		
C	Acrylamide	500			5
A	Actidione, Upjohn U-4527	60	-		
A	Actinomycin, NRRL 3764-28 E	14		0	
A	Actithiazic acid, Pfizer PA-95	60/14		8	7
M	Adenine sulfate	1000	+		0
SGS	Adenosine	500	-		
C&SA	Adonitol	500	+		-13
AA	dl-Alanine	1000	-		
C	Allantoin	500	-		
E&G	Allyl acetate	500	-		
S,M,AC	Allyl isothiocyanate	500/14		5	48
E&G	Allyl propionate	500	-		-5
Pl. ex.	Aloins from Aloe vera L., W. E. Weller	60	+		0
A	Amicetin, Upjohn, U-4761 [b]	60	+		6
AC,S	2-Aminobenzenethiol, Zn derivative, Hoffmann-LaRoche Ro 2-1009	200	+		55
AC,S	2-Aminobenzenethiol, Hg derivative, Hoffmann-LaRoche Ro 2-1136	120	+		23
C	p-Aminobenzoic acid	1000	-		
C	2-Amino-2-methyl 1,3-propanediol	500			-11
C	5-Amino-2-naphthol-HCl	500	+		10
M	p-Aminophenol	500			37
AC	p-Aminosalicilic acid hydrazide, Nepera	60	-		
A	Amphomycin, Na-salt, Bristol Lot 129	60/14	-	0	
C	Amyl alcohol	500	-		
C	iso-Amyl ether	500	-		
E&G	n-Amyl-n-butyrate	500	-		
A	Anisomycin, Pfizer OM 19312	60	-		

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
A	Antistine-HCl, CIBA	60	+		
C	Anthracene	500	-		
Su	l-Arabinose	500	-		
Su	d-Arabinose	500	-		
AA	l(+)-Arginine	1000	-		
C	Ascorbyl palmitate	500	+		27
C	Ascoridole	500	+		38
A	Ascosin, Commercial Solv. Lot E3	60	+		0
AA	dl-Aspartic acid	500	+		-25
A	Aspergillic acid, Squibb	14		0	
A	Aureolic acid, Abbott PA 44-765	60/14		43	55 to 0[c]
C	Aurin tricarboxylic acid	500	+		3
A	Azaserine, Parke Davis 2242 x 24P[b]	60	+		0
C	Azobenzene	500	+		13
A	Bacillomycin, Wyeth Lot 102-2	60	-		
A	Bacitracin, Pennick	14		0	
A	Bacitracin methylene disalicilate (DPS), Pennick	60	+		-5
C	Benzaldehyde	500	+		3
C	Benzidine sulfate	500	+		-12
AC	Benzoic acid	500	+		-6
M	Benzoyl peroxide	1000			26
C	Benzyl α-naphthyl ether	500	-		
C	Benzyl n-butyrate	500	+		10
AC	Benzyl p-hydroxybenzoate (Benzyl Parasept), Heyden	500	+		17
AC	Benzyltrimethylammonium chloride	1000			20
C	Betaine-HCl	500	-		-35
C	Bilirubin	500	-		
C	Biliveridin	500	+		4
AC	Bithionol, Sterling-Winthrop Win 4257	500			37[d]
A	Borrelidin, Na-salt, Hoffmann-La Roche	60	+		-6
A	Bostricoidin, F.R. Cajori, U. Colo.	60	+		9
C	Brucine alkaloid	500	+		-10
A	Burdock, Antibiotic from, Sterling Winthrop Res. Inst.	14		0	
M,AC	Butadiene monoxide	1200			14
E&G	Butyl acetate	500	-		
C	n-Butyl adipate	500	+		9
E&G	n-Butyl-n-butyrate	500	-		
M,AC	Butylene oxide S, Dow	2000			38
M,AC	Butylene oxides M, Dow	1000			4
M	Butyl hydroperoxide	1000			40±

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
AC	n-Butyl parahydroxybenzoate (Butyl Parasept), Heyden	500	+		14
S,AC	n-Butyl thiolsulfinate, Ster. Win.	1000		32	49
A	iso-Butyl vanillate, Squibb SF203	1500	+		-4
AC	sec. Butyl vanillate, Squibb MB650	500			-1
FA	n-Butyric acid	500	-		
Dis	Calcium hypochlorite	500			7
A	Candicidin, Pennick 1893 LMF	60	+		-5
FA	n-Capric acid	500	+		13
FA	n-Caproic acid	500	-		
C	Capryl alcohol	500	+		-4
FA	n-Caprylic acid	500	-		
A	Carbomycin, Pfizer OM 19312	60	+		6
	Cardelmycin, see Cathomycin				
A	Catenulin, Pfizer	60/14		15	20
A	Cathomycin, Na-salt, Merck 55R1547	60	+		0
A	Celesticetin salicylate, Upjohn U-4819	60	+		0
A	Cephalosporin N, N.G.Heatley, Oxford[e]	60			0
A	Cephalosporin P-1, N.G.Heatley, "	60	+		14
AC	Cetyltrimethylammonium bromide	500	+		74
M	Chloroacetone[f]	500			67
A	Chloramphenicol, Parke Davis	60/14		10	0
A	Chloramphenicol, p-methyl sulfonyl analog, Parke Davis Rx 172759 (CID-545)	60	+		0
A	Chloramphenicol analog, Hoffmann-La Roche Ro 1-6600	60	+		0
AC	p-Chlorophenoxyacetic acid	500			-25
AC	3-p-chlorophenyl-5-methyl rhodanine, Stauffer N-244	60	+		14
A	Chlortetracycline, Lederle	14		-6	
C	Choline chloride	500	-		
C	Cinnamate, sodium	500	-		
A	Cinnamycin acetate, NRRL	14		11	
A	Cinnamycin + 2nd active component, NRRL	60/14		32,3	12
A	Circulin sulfate, Upjohn U-4762	14		0	
C	Cinnamaldehyde	500	+		34
C	Citric acid	500	+		-65
A	Citrinin, WRRL	14		0	
A	Clavicin, Boots Drug Co.	14		-7	
Pl. ex.	Cloves, oil of	500	+		0

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
M	Cobaltous nitrate	5000			12
A	Colupulone, WRRL	60/14		18	12
A	Comirin, Colonial Microb.Res.Inst.	14		0	
M	Coumarin	500			-1
C	Creatine	500	-		
A	Crepin, N. G. Heatley, Oxford	60	-		
M	Cyclohexanol	500			-10
A	Cycloserine, Commercial Solv.Lot 24	60	-		
AA,S	l(+)-Cysteine-HCl	500	+		-10
A	Cytosamine, Upjohn U-6070	60	-		
C	Decahydronaphthalene	500			0
C	Decyl aldehyde	500	+		0
M	Deoxycholic acid	500			14
C	Diacetin	500	-		
M	s-Dichloroacetone	500			-14
C	1,8-Diaminonaphthalene	500	+		0
C	3,5-Diaminobenzoic acid	500	-		
C	4,4'-Diaminobenzophenone	500	-		
C	1,4-Diaminobutane dihydrochloride	500	-		
C	Dibutyl phthalate	500	+		3
M,AC	Diepoxybutane, WRRL	800			>77
AC	β-Diethylaminoethyl fencholate, Merrell	60	-		
S	Diethyldisulfide	330			36
M,S	Diethyldithiocarbamate, Na-salt	1000	+		56
C	Dihydroxystearic acid	500	-		
A	Dihydroquercetin	14		0	
A	Dihydrotomatadine, ERRL	14		0	
C	2,4-Dihydroxyacetophenone	500	+		1
C	p-Dimethylaminobenzaldehyde	500	+		6
C	Dimethyldihydroresorcinal	500	-		
M	Dimethyl sulfate	500			-20
C	2,4-Dinitro-l-naphthol-7-sulfonic acid	500	+		-2
C	Diphenylbenzidine	500	-		
C	Diphenylcarbamine chloride	500	+		11
C	s-Diphenylcarbazide	500	+		0
SGS	Dipicolinic acid	500	-		
M,S	Dithiocarbamic acid, Na-salt	1000			32
C	Disodium phenyl phosphate	500	-		
C	Dodecylguanidine-HBr,Sterling Winthrop Win 341 HrB 87	500	+		86
C&SA	Dulcitol	1000	-		
A	Duramycin, NRRL 4307-54-A	180			-4
A	Elaiomycin, Parke Davis 2337 x 1	60	+		13
A	Endomycin, Upjohn U-4568	14		0	
C	Epichlorohydrin	500	-		

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
C	Ephedrine	500	-		
M,AC	9,10-Epoxystearamide, ERRL	1000			13
M,AC	cis-9,10-Epoxystearic acid, ERRL	1000			22
M,AC	trans-9,10-Epoxystearic acid, ERRL	1000			25
A	Erythromycin, Lilly	60/14		10	0
C&SA	Esculin	1000	-		
A	Etamycin base, Bristol Lot 165	60	+		14
C	Ethyl adipate	500	+		9
So	Ethyl alcohol	1%			-8
C	Ethyl bromopropionate	500	-		
M	Ethyl-N-n-butyl carbamate	1000			2
M	Ethyl-N,N-di-n-butyl carbamate	500			4
E&G	Ethyl-n-butyrate	500	-		
C	Ethyl chloroacetate	500	-		
S,AC	s-Ethyl-l-cysteine, L. E. Weller, Michigan State	60	-		
C	Ethylene diacetate	500	-		
So	Ethylene glycol	500	-		
M,AC	Ethylene oxide	670			80
C	Ethyl glycolate	500	-		
AC	Ethyl p-hydroxybenzoate (Ethyl Parasept), Heyden	60	-		
AC,S	Ethyl isothiocyanate	500			42
C	Ethyl lactate	500	-		
E&G	Ethyl laurate	500	+		0
M	Ethyl-n-nitroso-N-methyl carbamate	500			0
E&G	Ethyl oleate	500	-		
E&G	Ethyl stearate	500	-		
S	Ethyl thiocyanate	500			7
C	Ethyl tartrate	500	-		
A	Ethyl vanillate, Squibb Inst.SF 146	60	-		
C	Ferrous chloride	2000			-55
A	Filipin, Upjohn U-5956	60	-		
A	Flavone (synthetic), L. E. Weller, Michigan State	60	+		4
C	Folic acid	500	-		
M,Dis	Formaldehyde [b]	500			83
A	Fradicin, Commercial Solv.	60	+		0
Su	Fucose	1000	-		
A	Fumagillin, Upjohn	14		0	
AC	Furazolidone, Eaton	14		0	
C	Gallic acid	500	-		
Pl.ex.	Garlic oil, Florasynth Labs.	1000/100		10	52

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
Pl.ex.	Garlic powder	1000		5 [g]	2
AC, Dye	Gentian violet [d]	500	+		35
AC	Giberellin A and Giberellic acid (mixture), NRRL 1956-68-B	180			-10
A	Gliotoxin, Squibb	60	-		
C	Glucosamine-HCl	500	-		
S	Glutathione isomer, WRRL	500	-		
AA	d-Glutamic acid	1000	-		
C	Glutaric acid	500	-		
C	Glycerol α-monochlorohydrin	500	+		21
C	Glycerol sorbitan laurate, Atlas G-672	500	+		10?
C	Glycerol trichlorohydrin	500	-		
A	Grifolin, Y. Hirata, Nagoya Univ.	14		0	
A	Grisein, Merck	60	-		
A	Griseoviridin, Parke Davis 2069 x 9c	60	+		13
C	Guaicol	500	-		
C	Guanidine-HCl	500	-		
C	Guanine-HCl	500	-		
A	Helixin A, C. Leben, U. Wisconsin	60/14		11	1
A	Helixin B, " "	14		0	
C	Hemin	500	+		15
C	n-Heptamide	500	-		
FA	n-Heptylic acid	500	-		
C	Hexadecanol	500			0
A	Hexahydrocolupulon, WRRL	60/14		17	10
C	Hexamethylenediamine	700			-15
AC	Hexylresorcinol	1000			48
C	Hippurate, sodium	1000	-		
A	Humulone, WRRL	60/14		11	9
C	Hydantoin	500	-		
C	Hydrocinnamaldehyde	500	+		24
M, Dis	Hydrogen peroxide [b]	200	+		83
M	Hydroquinone	500			26
C	β-Hydroxyethyl acetate	500	-		
C	α-Hydroxyphenazine	500	+		12
M, AC	8-Hydroxyquinoline sulfate	500	+		9
A	Hydroxystreptomycin, Abbott	60	+		4
A	Hygromycin, Lilly	60/14		30,4	0
A	Illudin M, Robbins, N.Y. Bot. Garden	60	+		10
A	Illudin S, " " " "	60	+		25
C	Indole	500	+		6

Table 2 - cont'd.

Class	Substance	Conc.[a/] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
AC	Indole-3-N-butyric acid	100		4	
AC	Indole-3-propionic acid	60/100		12	4
C&SA	Inulin	1000	-		
AA	dl-Isoleucine	1000	-		
AC	Isonicotinic acid hydrazide	500			
C	Itaconic acid	500	-		
AC	Kojic acid	500	-		
C	Lactic acid	500	-		
A	Laterosporin A, NRRL	25/14	+	10	-8
A	Laterosporin B, NRRL	60	+		7 ?
FA	Lauric acid	500	+		30
C	Lauric aldehyde	500	+		10
AA	dl-Leucine	1000	-		
A	Licheniformin A.5, Kent, Ministry of Supply, England	14		0	
C	Linalyl acetate	500	-		
M	Lithium chloride	1000			-35
Su	d-Lyxose	500	-		
	Lupulone, See Colupulone				
AC	Lysozyme, WRRL	500	-		
C	Maleic acid	500	-		
C	l-Malic acid	500	+		-6
A	Malucidin, Naugatuck Chemical	6000	-		
M	Manganese chloride	2000			-40
C&SA	Mannitol	1000	-		
A	Mannosidostreptomycin, Squibb	60	+		0
A	Mantomide, Sterling-Winthrop Win 5047	60	-		
A	Marasmic acid, Robbins, N. Y. Bot. Garden	60	-		
A	Marsilid, Hoffmann-La Roche	60	-		
Pl. ex.	Menispermum canadense, alkaloids of, L. E. Weller, Michigan State	60	-		
Dis	Merthiolate	1000			8
AC	Methenamine mandelate, Nepera	60	-		
AA,S	dl-Methionine	1000	-		
C	p-Methoxy-tert-amylbenzene	500	+		5
A	5-Methoxytoluquinone, Robbins, N. Y. Bot. Garden	60	-		
C	Methyl-p-aminobenzoate	500	+		0
C	Methyl benzoate	500	-		

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
C	Methyl-o-benzoylbenzoate	500	+		4
C	Methyl-m-bromobenzoate	500	+		7
C	Methyl-o-bromobenzoate	500	+		7
C	Methyl-p-bromobenzoate	500	+		8
E&G	Methyl-n-butyrate	500	-		
C	Methyl-o-chlorobenzoate	500	+		13 ?
C	Methyl-3,5-dibromoanthranilate	500	-		
C	Methyl-α,β-dibromopropionate	500	-		
S,M,AC	N-Methyldithiocarbamate dihydrate Na-salt, Stauffer N-869 [h]	170	+		32
M,Dye	Methylene blue chloride	100			30 [d]
C	α-Methyl-d-glucoside	1000	-		
AC	Methyl-p-hydroxybenzoate, Heyden	500	-		
C	dl-Methylmalonate	500	-		
AC	2-Methyl-1,4-naphthoquinone (Menadione, Vitamin K_4) Parlam Corp.	500	+		76
C	Methyl-p-nitrobenzoate	500	+		6
C	Methyl-m-nitrobenzoate	500	-		
C	Methyl-o-nitrobenzoate	500	-		
A	Methylol gramicidin, WRRL	60/14		8	-5
C	Methyl phenylacetate	500	-		
E&G	Methyl oleate	500	-		
E&G	Methyl palmitate	500	-		
E&G	Methyl nononate	500	-		
C	Methyl salicylate	500	-		
C	Methyl sebacate	500	+		11 ?
E&G	Methyl stearate	500	-		
E&G	Methyl undecylenate	500	-		
AC,Dye	Methyl violet	500	+		18
A	Methymycin, Squibb M2140 HL-102	60	+		10
A	Micrococcin, N. G. Heatley, Oxford	60	+		7
C	Monoacetin	500	-		
C	Monochloracetic acid	500	-		
C	Monopropionin	500	-		
A	Mycomycetin, Abbott E-4485 NA 269-M2	60	-		
A	Mycostatin, Squibb 52282-023	60	-		
A	Mycosubtilin, Merck 9R7098	60	-		
FA	Myristic acid	500	+		33
M	1-Naphthol	500			11
C	1,2-Naphthoquinone-4-sulfonic acid	500	+		14

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
C	Naphthoresorcinol	500	+		7
M	α-Naphthylamine	500			10
A	Neomycin, Commercial Solv.	60/14		10	9
A	Neomycin A, Upjohn U-5214	60	+		1
A	Neomycin B, Upjohn U-4567	60	+		17
A	Netropsin, Pfizer	60	-		
A	Nigericin, Commercial Solv.	14		-13	
AC,S	Nicotinaldehyde thiosemicarbazone Hoffmann-La Roche Ro 1-5631	60	+		0
A	Nisin, Benger Ltd., England	40/14		56	74 [d]
AC	Nitrofurantoin, Eaton	500/14		20	0
AC	Nitrofurfuryl methyl ether, Eaton	14		0	
C	Nitron	500	+		-10
C	Nonyl aldehyde	500	+		
AC	Nordihydroguaiaretic acid, Stange	500	+		63
C	n-Octadecanol	500	-		
C	n-Octyl alcohol	500	-		
FA	Oleic acid	500	+		17
Pl. ex.	Olive extract, rich in bitter glucoside, Cruess, Univ. of Calif.	5000			-23
A	Oxamycin, Ca-salt, Merck 55R2172	60	-		
A	Oxyaspergillic acid, Squibb	60	-		
AC	Oxystarch, periodate 100%, WRRL	500			16
A	Oxytetracycline, Pfizer	14		-6	
FA	Palmitic acid	500	+		34
C	Para-butyraldehyde	500	-		
A	Penicillic acid, NRRL	60	-		
A,M	Penicillin	60/14		10	-1
C	Pectic acid	500	-		
C	Phenanthrene	500	-		
AC	Phenoxyethyldimethyldodecylammonium bromide (Bradosol), CIBA	500	+		80
C	p-Phenylacetophenone	500	-		
C	n-Phenylanthranilic acid	500	+		0
M	p-Phenylenediamine	500			-25
S	Phenyl isocyanate	500			-4
S,AC	Phenyl isothiocyanate	500			46+
C	2-Phenylquinoline-4-carboxylic acid	500	-		
S	Phenylthiohydantoic acid	500	-		
C	Phenyl acetate	500	-		
A	Phloretin, Dept. Agr., Kentville, N. S., Canada	60	-		
A	Phagolessin A58, Robbins, N. Y. Bot. Garden	60	-		

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
C	Phlorglucin	500	-		
C	Phytosterol	500	-		
C	Picolinic acid	500	+		-12
A	Picromycin, Parke Davis 2124 x 36	60	+		0
AC	Pivalizid, Nepera	60	-		
A	Pleocidin, Sharp and Dohme	60/14		12	-8
A	Pleomycin, Sharp and Dohme	60	+		-8
A	Pleuromutilin, Robbins, N. Y. Bot. Garden	60	+		-10
AC	Polylysine-HCl, n=33) Stahman,	14		3	
AC	Polylysine-HCl, n=83) U. Wisconsin	14		7	
A	Polymyxin D-HCl, Commercial Solv.	14		5	
A	Polypeptin, NRRL 3764-107C	14		0	
Dis	Potassium permanganate	500	-		
C	iso-Propanolamine	500	-		
C	Propenyl guaethol	500	-		
M,AC	β-Propiolactone	3000	+		68
AC	Propionate, Na Salt	1000	-		
C	n-Propyl adipate	500	+		0
E&G	n-Propyl-n-butyrate	500	-		
S	n-Propyl disulfide	500			30
C	Propylene chlorohydrin	500	-		
So	Propylene glycol	1%			-13
C	Propylene glycol dipropionate	500			2
M,AC	Propylene oxide	2000			74
C	n-Propyl gallate	500	+		-13
AC	Propyl-p-hydroxybenzoate, Heyden	60	-		
C	Phthalimide, potassium salt	500	-		
A	Puberulic acid, Raistrick	60	-		
A	Puberulonic acid, Raistrick	60	-		
A	Puromycin, Lederle Lot 7-9605	14		-3	
A	Puromycin analog 2484L, Lederle	60	-		
AC	Pyribenzamine-HCl, CIBA	60			-3
S,AC	3-Pyridinethiol, Zn-derivative, Hoffmann-La Roche Ro 2-1485	200	+		52
S,AC	Pyradinethione, Cu-salt, Squibb	500	-		
S,AC	Pyradinethione, Na-salt, Squibb	500	-		
M	Pyrogallol	500	+		-13
A	Quercetin	500			6
C	Quinaldine	500	+		1
C	Quinine sulfate	500	+		-11
C	Quinizarin-2-sulfonic acid	500	+		5

Table 2 - cont's

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
M	Quinoline [b]	500	-		
C	Quinolinic acid	500	-		
Su	Raffinose	500	-		
Dye	Resazurin	100	-		
A	Resistomycin, Schenley	60	-		
C	Resorcinol	500	-		
A	Rhodomycetin, S. A. Waksman	60/14		15	9
A	Rimocidin sulfate, Pfizer Lot 52951	14		2	
A	Rutin	60	-		
C	Saccharin	1000	-		
C&SA	Salicin	1000	-		
C	Salicylate, sodium	500	-		
AC	Salizid, Nepera	60	-		
C	Semicarbazide-HCl	500	-		
Dis	Silver nitrate	170			52
Dis	Sodium bisulfite	2000	+		-33
AC	Sodium diacetate	500	-		
C	Sodium nitrate	1%	+		-75
AC	Sodium nitrite	3000	+		41
Pl.ex.	Sodium Palconate, Pacific Lumber Co. (an extract of redwood bark)	500	-		
AC	Sorbic acid	14		0	
C&SA	d-Sorbitol	500	-		
C	Span 20 (Sorbitan monolaurate), Atlas	500	+		7
C	Span 80 (Sorbitan monooleate), Atlas	500	+		-4
A	Spinulosin, Raistrick	500	-		
A	Spiramycin sulfate, Merck 55R1089	60	+		-3
FA	Stearic acid	500	+		34±
A	Streptogramin, Sharp and Dohme 900-R-223	60/14		9	9
A	Streptogramin, Merck 55R2054	60	+		-2
A	Streptolin, Peterson, U. Wisconsin	60	+		-5
A	Streptolydigin, Na salt, Upjohn U-5482	60	+		9
A	Streptomycin-$CaCl_2$ complex, Merck	14		0	
	Streptonivicin, see Cathomycin				
A	Streptothricin-HCl, Merck 4R3256	14		0	
A	Subtenolin, NRRL 2905-108-A	60/14		11	0
A	Subtilin, WRRL, Lot 326	20		53	85 [d]
A	Subtilin A, A166-161-220, WRRL	8			72 [d]
A	Subtilin B, A166-22-70, WRRL	8			25 [d]

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
A	Subtilin methyl ester, WRRL 21 Methoxyl = 5.1 equiv./10^4 g.	7			81 [d]
A	Subtilin methyl ester, WRRL 36 Methoxyl = 10.9 equiv./10^4 g.	60/14		50	70 [d]
C	Sulfanilic acid	500	-		
C	Sulfasalicylic acid	500	-		
C	Sulfolane, Shell Development	500			-2
C	3-Sulfolene, Shell Development	500			-4
A	Synkamin, Parke Davis	60			-8
A	Synnematin B, Michigan Dept. Health [e]	60	-		
AC	Tannic acid	500			38
C	Taurine	500	-		
A	Terreic acid, Bristol Lot 966-14	60	-		
M	Tetrachlorohydroquinone	500	+		65
C	Tetrachloroquinone (Chloranil)	500	+		-3
A	Tetracycline-HCl, Lederle	60	+		2
C	Tetraethylammonium bromide	500	-		
C	Tetrahydrofurfuryl maleate	500	-		
C	Tetramethylammonium bromide	500	-		
Dye	Tetrazolium blue	100	-		
C	Theobromine	500	-		
A	Thioaurin, Sharp and Dohme	60	+		-19
A	Thiocymetin, Sterling Winthrop, Win 5063-2	60	+		10
A	Thiocymetin-like antibiotic, Sterling Winthrop Win 5094-2	60	+		13
A	Thiolutin, Pfizer 882-102-6 [b]	14		3	
A	Thiolutin-like antibiotic, Sharp and Dohme, Antibiotic 786	60			0
AC,S	Thiosemicarbazone of 3-indole carboxaldehyde, Weller, Michigan State	60	-		
S	Thiourea	500	-		
C	Tiglaldehyde	500	-		
A	Tomatadine, ERRL	14		0	
A	Tomatine, ERRL	14		0	
Su	Trehalose	500	-		
C	Tributyl citrate	500	+		-2
C	Tributyl phosphate	500	+		19
E&G	Tricaproin	500	-		
C	Triketohydrindene hydrate	500	+		38

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
E&G	Trimyristin	500	-		
E&G	Tripalmitin	500	-		
E&G	Tripropionin	500	-		
E&G	Tristearin	500	-		
C	dl-Tropic acid	500	-		
C	Tween 20 (Polyoxyethylene sorbitan monolaurate), Atlas	500	-		
C	Tween 80 (Polyoxyethylene sorbitan monooleate), Atlas	500	+		3
A	Tyrothricin, Merck R89 43571	14		>4	
C	Undecanol	500	+		13 ?
C	Undecylenic aldehyde	500	+		17
C	Uracil	1000	-		
C	Urea	500	-		
M	Urethane	1000			-11
C	Uric acid	500	-		
A	Usnic acid, WRRL	60/14		-20	5
A	Ustilagic acid, Nat. Res. Council Canada	60	-		
C	iso-Valeraldehyde	500	-		
C	Vanillin	500	-		
A	Viomycin, CIBA	60	+		9
A	Viridogriseim, Parke Davis 5840xIJJJ	60	+		11
AC	Vitamin K_1	500			0
AC	Vitamin K_4. See 2-Methyl-1,4-naphthoquinone				
AC	Vitamin K_5	200		0	
A	Vivicil, Schenley	60	-		
A	Vulpinic Acid, WRRL	14		0	
A	Xanthomycin, Parke Davis 868x88	60	-		

Phenylamines submitted by M. C. Kloetzel, University of Southern California:

AC	4-Amino-4'-nitrodiphenylamine	60	-		
AC	4-Bromo-N-methyl-4'-nitrodiphenylamine	60	-		
AC	4-Bromo-2'-nitrodiphenylamine	60	-		
AC	2-Chloro-4'-nitrodiphenylamine	60	+		0
	2-Chloro-4'-nitrodiphenylamine	500			-1
AC	N-Methyl-2,4-dinitrodiphenylamine	60	-		
AC	N-Methyl-4,4'-dinitrodiphenylamine	60	-		
AC	N-Methyl-4'-nitrodiphenylamine	60	+		-9

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
AC	2-Methoxy-2'-nitrodiphenylamine	60	-		
AC	4-Nitrosodiphenylamine	60	-		
AC	N-Nitrosodiphenylamine	60	+		0
	N-Nitrosodiphenylamine	500			35
	Substances prepared by A. Furst, Stanford Medical School, for screening against certain tumors and viruses:				
AC	45 substances	60	all -		
AC	10 substances	60	all +		+9 to -15
	Aromatic phosphinic and phosphonic acids supplied by J. D. Thayer, U.S. Public Health Service:				
AC	49 substances	60	all -		
A	Bristol 1-720	60/14		19	4
A	Bristol 2-800	60/14		7	-8
A	Bristol 6-850	60	+		0
A	Bristol A8506-65	60	+		19
AC	Dow DHA-S, P 30-4-910	500	-		0
C	Griffiths Lab., Anti-Oxidant G-5	500	+		10?
A	Hoffmann-LaRoche Antibiotic X-206	60	+		0
A	Hoffmann-LaRoche Antibiotic X-464	60	+		8
A	Hoffmann-LaRoche Antibiotic X-537A	60	+		0
A	Lilly 05865	60	+		12
A	Lilly M17365	60	+		7
A	Merck 52 R 4009	14		-3	
A	Merck 52 R 5989	14		-7	
A	Merck 52 R 6107	14		5	
A	Merck 52 R 6763	14		5	
A	Merck 53 R 642	60/14		6	-8
A	Merck 53 R 643	60/14		11	-4
A	Merck 53 R 644	60/14		6	3
A	NRRL Antibiotic 136	60	+		5
A	NRRL Antibiotic EF 185	60	+		8
A	NRRL Antibiotic H-36, 3903-150-1	60	-		
A	NRRL Antibiotic F-12 culture filtrate, undiluted		+		4
A	NRRL Antibiotic F-14 culture filtrate, undiluted		+		-18
A	NRRL Antibiotic F-17 culture filtrate, undiluted		+		- 4
A	NRRL Antibiotic F-17 butanol extract	60	+		0

Table 2 - cont'd.

Class	Substance	Conc.[a] ppm.	Presence of growth Incubation test	Reduction of D value, % Thermoresistometer	Reduction of D value, % Counting test
A	NRRL Antibiotic F-18 culture filtrate, undiluted		-		
A	NRRL Antibiotic F-73 culture filtrate, undiluted		+		-12
A	NRRL Antibiotic F-416 culture filtrate, undiluted		+		-3
A	NRRL Antibiotic F-566 culture filtrate, undiluted		+		7
A	Pfizer PA-122	60	+		0
A	Pfizer No. 1	60/14		12	-2
A	Pfizer No. 6	60/14		8	-2
A	Pfizer No. 4	14		7	
A	Pfizer No. 13	60/14		8	2
A	Pfizer No. 22	60/14		8	0
A	Pfizer Nos. 3,5,7,8,9,10,11,12, 14,16,21,24,27	14		all < 6	
M,AC	Shell Dev. Epon 562 (epoxide)	1000			14
M,AC	Shell Dev. Epon 828 (epoxide)	1000			4
AC	Shell Dev. P-10190 (C_{20} dibasic unsaturated acids)	500			26
AC	Shell Dev. P-10280 (C_{20} dibasic saturated acids)	500			24
AC	Sterol Derivatives, Inc. "Fungus Mold Inhibitor"	1000	-		

Footnotes to Table 2

a/ When two concentrations are given (e.g., 60/14), the second applies only to the thermoresistometer test.

b/ Of the substances listed here as non-mutagenic, Szybalski (7) recently reported azaserine to be mutagenic and amicetin and thiolutin to have weak or doubtful mutagenicity against a strain of Escherichia coli. Of those listed here as mutagenic, he found quinoline to be non-mutagenic and formaldehyde and hydrogen peroxide to have weak or doubtful mutagenicity.

c/ Early results with aureolic acid showing large reductions in D value could not be confirmed in later experiments.

d/ Substances thus designated were present in the counting plate in sufficient concentration to affect outgrowth of the surviving spores. Hence their activity may be that of killing spores after, not during, the heat treatment. (See Michener et al, 6.) Subtilin is known to kill the spores at the time they germinate (Michener, 4).

e/ Cephalosporin N and synnematin B are identical (E.P. Abraham et al., 1).

f/ Because of its toxicity, chloroacetone was not investigated further although it greatly reduced the D value.

g/ Saturated water extract.

h/ This concentration was more active than 500 or 1000 ppm.

Table 3. Sources from which Samples were Obtained

Abbott Laboratories, North Chicago, Illinois

Atlas Powder Co., Industrial Chemicals Dept., Wilmington 99, Delaware

Benger, Ltd., Holmes Chapel, Cheshire, England. Nisin is now obtainable from Aplin and Barrett, Ltd., Newton Road, Yeovil, Somerset, England.

Boots Drug Co., Nottingham, England

Bristol Laboratories, Syracuse 1, New York

CIBA Pharmaceutical Products, Inc., Summit, New Jersey

Dr. F. A. Cajori, Department of Medicine, University of Colorado, Denver 20, Colorado

Colonial Microbiological Research Institute, Port-of-Spain, Trinidad

Commercial Solvents Corporation, Terre Haute, Indiana

Dr. W. V. Cruess, Dept. of Food Technology, University of California, Berkeley, California

Dow Chemical Company, Midland, Michigan

Eaton Laboratories, Division of Norwich Pharmacal Co., Norwich, N. Y.

ERRL: Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pa.
Antibiotics: C. P. Krewson, Chemist, Biochemical Investigations.
Epoxides: D. Swern, Supervisory Chemist, Animal Fats Laboratory

Dr. Arthur Furst, Stanford University School of Medicine, San Francisco 15, California

Florasynth Laboratories, Inc., 900 Van Nest Ave., New York, New York

Griffith's Laboratories, 1415 W. 37th Street, Chicago 9, Illinois

Dr. N. G. Heatley, Sir William Dunn School of Pathology, University of Oxford, England

Dr. Y. Hirata, Nagoya University, Nagoya, Japan

Heyden Newport Chemical Corporation, 342 Madison Avenue, New York 17, New York

Hoffman-LaRoche, Inc., Roche Park, Nutley 10, New Jersey

Department of Agriculture, Experimental Station, Kentville, N. S., Canada
C. J. Bishop, Superintendent

Dr. L. H. Kent, Ministry of Supply, Porton, England

Dr. M. C. Kloetzel, Department of Chemistry, University of Southern California, Los Angeles 7, California

Dr. C. Leben, College of Agriculture, University of Wisconsin, Madison 6, Wisconsin

Lederle Laboratories Division, American Cyanimide Co., Pearl River, New York.

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Ind.

The Wm. S. Merrell Co., Cincinnati, Ohio

Merck and Company, Inc., Rahway, New Jersey

Michigan Department of Health, Division of Laboratories, Lansing 4, Michigan
Dr. B. H. Olson, Chief

National Research Council, Ottawa, Canada

Naugatuck Chemical Division, U.S. Rubber Co., Naugatuck, Conn.

Nepera Chemical Co., Inc., Nepera Park, Yonkers 2, New York

Wm. J. Stange Co., 342 N. Western Ave., Chicago 12, Illinois

NRRL: Dr. R. W. Jackson, Head, Fermentation Division, Northern Utilization Research & Development Division, Agricultural Research Service, U.S. Dept. of Agriculture, Peoria, Illinois

Pacific Lumber Company, 100 Bush Street, San Francisco, California

Parlam Corporation, 340 Canal Street, New York 13, New York

Parke, Davis and Company, Research Division, Detroit 32, Michigan

S. B. Penick and Company, Research Division, 50 Church St., New York 8, N.Y.

W. H. Peterson, University of Wisconsin, Madison 6, Wisconsin

Charles Pfizer and Co., Inc., 630 Flushing Ave., Brooklyn 6, New York

Dr. Harold Raistrick, London School of Hygiene and Tropical Medicine, London, England

Dr. W. J. Robbins, New York Botanical Garden, New York 58, New York

Schenley Laboratories, Inc., 350 Fifth Ave., New York 1, New York

Sharp and Dohme Division of Merck and Co., Inc., Research Laboratories, West Point, Pennsylvania

Shell Development Co., Emeryville 8, California

The Squibb Institute for Medical Research, New Brunswick, New Jersey

Dr. M. A. Stahman, College of Agriculture, University of Wisconsin, Madison 6, Wisconsin

Stauffer Chemical Co., 1496 E. Fremont Rd., Mountain View, California

Sterling-Winthrop Research Institute, Rensseleer, New York

Sterol Derivatives, Inc., 2636 Medford St., Los Angeles 63, California

Dr. J. D. Thayer, Venereal Disease Experimental Laboratory, U.S. Public Health Service, P. O. Box 1049, Chapel Hill, North Carolina

The Upjohn Company, Kalamazoo, Michigan

Dr. L. E. Weller, Department of Agricultural Chemistry, Michigan State College, East Lansing, Michigan

WRRL: Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany 10, Calif. vulpinic acid and oxystarch, F. DeEds; butadiene diepoxide, W. G. Rose; glutathione isomer, M. K. Walden; usnic acid, J.B. Stark; lysozyme, G. Alderton; other WRRL entries, J. C. Lewis

Dr. S. A. Waksman, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

Wyeth Institute for Medical Research, Depts. of Virology and Bacteriology, Philadelphia 1, Pennsylvania

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1. Abraham, E. P., Newton, G. G. F., Olson, B. H., Schuurmans, D. M., Schenck, J. R., Hargie, M. P., Fisher, M. W., and Fusari, S. A. 1955. Identity of cephalosporin N and synnematin B. Nature, 176: 551.

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